A STUDY OF SHIGELLA CULTURES ISOLATED IN INDIA AND BURMA, WITH SPECIAL REFERENCE TO TWO PREVIOUSLY UNDESCRIBED SERO-TYPES

BY

CHARLES DONALD COX

B.S., University of Illinois, 1940
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AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN BACTERIOLOGY
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UNIVERSITY OF ILLINOIS, 1947

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After Shiga (1898) first described a bacillus as being the cause of 36 cases of dysentery in Japan reports frequently appeared from many parts of the world concerning bacilli associated with human dysentery. The literature soon became cluttered with many descriptions of dysentery bacilli, some of which were thought to be identical at the time. However, it became apparent that many criteria used in separating these organisms, especially some carbohydrate reactions, were inconstant. Andrewes (1918) contributed much to the classification of these organisms by recognizing lactose and mannitol as critical carbohydrates in their separation and observing that classification by carbohydrate reactions did not always parallel serological classification.

More stress was placed upon serological identification of the dysentery bacilli after Andrewes' (1918) paper, and later studies by Andrewes and Inman (1919), Boyd (1931, 1932, 1935, 1936, 1938, 1940), Wheeler (1944a, b) and Weil, Black and Farsetta (1944) are largely responsible for our present line of thought concerning the separation and identification of these organisms.

New sero-types of Shigella are now appearing frequently from many parts of the world. This is undoubtedly due in part to the large numbers of men hospitalized with bacillary dysentery during World War II and more accurate bacteriological studies of these cases by simplified sero-typing procedures.

During the period from August, 1944, to October, 1945, the author isolated or identified 664 cultures of Shigella

largely from American military personnel in India and Burma. These cultures comprised the following types: Shigella dysenteriae (0.9%), Sh. ambigua (5.9%), Sh. sp. Sachs types Q771, Q902, Q1030 and Q454 (total of 3.8%), Sh. paradysenteriae Flexner V, W and Z (total of 25.2%), Sh. paradysenteriae Boyd types 103, P119 and 88 (total of 23.8%), Sh. paradysenteriae Boyd types 170, P288, P274, D1, D19 and P143 (total of 5.9%), Sh. sp. Manchester type (0.8%), Sh. sonnei (24.4%), Sh. dispar types I and II (total of 1.1%), Sh. alkalescens type II (0.5%), new sero-types (0.6%) and unidentified cultures possessing morphological and cultural characteristics of the genus Shigella (7.5%).

Several cultures were inagglutinable in known Shigella antisera at the time of isolation in India and Burma but were identified by more extensive tests in this country. However, many of these previously inagglutinable cultures remained unidentified after further investigation.

Forty such inagglutinable strains were returned to this country for further biochemical and serological tests. The main reasons for the biochemical investigations were to eliminate cultures possessing characteristics incompatible with commonly accepted characteristics of the genus Shigella and to group the cultures according to their ability to ferment lactose and mannitol. Such grouping facilitated later serological studies.

As a result of the more extensive investigations in this country 4 of the 40 cultures were discarded on the basis of motility and urease reactions and were not studied further.

Five cultures were serologically identified as Sh. ambigua, although all were inagglutinable in Sh. ambigua antiserum when first isolated. It is evident that some specific heat-stable factor was present in some strains isolated in India which masked or inhibited agglutination with Sh. ambigua antiserum. This factor deteriorated or disappeared upon prolonged cultivation on laboratory media rendering the

culture antigenically identical with Sh. ambigua. This generally supports previous work of Large and Sankaran (1934) and Boyd (1935). It is unfortunate that comparative antigenic studies were not made of these inagglutinable strains with typical Sh. ambigua strains upon isolation in India.

Three cultures were identified by agglutination and adsorption tests as biochemical variants of Sh. alkalescens type II. One of these did not ferment mannitol and contained alpha antigen. The other two fermented lactose and salacin.

One culture was identified as Sh. dispar type I, but as a non-heated antigen it failed to agglutinate to the titer of the antiserum.

Six cultures were serologically identified as Sh. dispar type II, although there was wide variation in their fermentation of lactose and sucrose. Two of the cultures fermented both carbohydrates, and two fermented neither carbohydrate. One culture gave variable fermentation of lactose but did not ferment sucrose, and the other culture showed variable fermentation of sucrose but did not ferment lactose. Adsorption studies involving these six cultures also brought out the antigenic similarity between Sh. alkalescens type III (Neter, 1944) and Sh. dispar type II (Carpenter, 1943, 1944). This is in agreement with other recent work by Carpenter and Stuart (1946) who showed Sh. alkalescens type III to be a sub-type of Sh. dispar type II.

Eighteen of the 40 cultures remained unidentified, and their taxonomic position as members of the genus Shigella seems questionable at this time. All 18 cultures fermented lactose, and 6 of the cultures fermented salacin. Four of the 18 cultures were lactose-negative, mannitol-negative upon original isolation in India and with two subsequent tests in this country, but three later tests have shown them to ferment mannitol with acid production within 24 hours and lactose with acid production within 6 to 10 days. Two other cultures were lactose-negative, mannitol-positive upon

original isolation and with two subsequent tests in this country, but four final tests showed them to ferment lactose to acid within 3 to 6 days. Four types of carbohydrate media were used and were checked with representative strains of the various fermentative groups of Shigella. It is not felt that the type of carbohydrate medium could be considered a likely cause of this change in fermentative ability. Such changes have previously been reported. These 18 cultures showed no antigenic relationship to any of the known members of the genus Shigella, although some confusing agglutination occurred which could not be explained by subsequent adsorptions.

Four cultures were found to comprise two previously undescribed sero-types. These consisted of one culture of sero-type 58, which belongs to the lactose-negative, mannitol-negative group, and three cultures of type 112, which belongs to the lactose-negative, mannitol-positive group of Shigella. The biochemical characteristics of the two new sero-types were consistent throughout. Type 58 fermented only dextrose, maltose, arabinose, sorbitol and xylose (slowly). It did not ferment lactose, mannitol, sucrose, dulcitol, rhamnose, raffinose, adonitol, inositol nor salacin and did not produce indol. In its ability to ferment xylose type 58 does not resemble other non-mannitol fermenting members of the genus Shigella. Biochemically sero-type 112 resembles Sh. sp. etousa (Lavington I) or some of the Sh. paradysenteriae Boyd types, although dulcitol was not fermented as in the case of most of the less frequently encountered Boyd types, i. e., Boyd's sub-group B comprising types 88, P288, D1, D19 and P274 (Boyd, 1932). Type 112 produced acid from dextrose, mannitol, arabinose, xylose, sorbitol and maltose (slowly). It did not ferment lactose, sucrose, dulcitol, rhamnose, raffinose, adonitol, inositol nor salacin and indol was not produced. No gas was produced by either type from any carbohydrate tested. Both types were Gram negative, non-motile, non-sporulating short bacilli, and neither type produced urease, utilized citrate as the sole source of carbon

or liquified gelatin.

Both types 58 and 112 showed little or no cross agglutination with any of the recognized Shigella types. Type 58 antiserum gave partial agglutination in low titers (1:40, 1:80) with antigens of some of the Flexner, dispar and alkalescens groups, but this crossing was considered insignificant. Antisera against two of the type 112 strains gave agglutination in low titers with antigens of Sh. paradysenteriae Boyd D19 and Sh. dispar type IV, and antiserum against the third strain of type 112 gave agglutination in low titer with antigens of Sh. paradysenteriae Flexner V, Flexner Y and Boyd D19 and Sh. dispar type IV. However, adsorption of type 112 antisera with crossing antigens demonstrated the absence of any major common antigens.

It is hoped that sero-types 58 and 112 may be compared serologically with other new sero-types as they may be reported in the future, or with such types which may not have been described in the literature. The reporting of new types or strains by their culture numbers is confusing, but assigning species names to such types is even less deirable and certainly unwarranted pending extensive comparative studies with other types or strains. Thus, sero-types 58 and 112 are left with their culture number designations until further investigations determine their proper place among the dysentery bacilli within the genus Shigella.

VITA

The author was born in Danville, Illinois, on September 10, 1918. He attended elementary school in Fithian, Illinois, and graduated from Oakwood Township High School in 1936. He received the B. S. degree from the University of Illinois in 1940 and the M. S. degree in 1941 from the same institution.

He was assistant in the Department of Bacteriology, University of Illinois, from September, 1940, to June, 1942, and from February, 1946, to June, 1947. From 1942 to 1946 he served as a bacteriologist in the United States Army. Two of these years were spent as bacteriologist for the Ninth Medical Laboratory serving the China-Burma-India theater of war. He was separated from active service as a Captain, Sn. S. in February, 1946.

Societies of which he is a member include American Public Health Association, Society of Illinois Bacteriologists, Sigma Xi and Phi Sigma.

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